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Vision Research 46 (2006) 678–687

Vision Research

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Contrast sensitivity of wildtype mice wearing diffusers or spectacle lenses, and the effect of atropine

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Received 25 February 2005; received in revised form 22 April 2005

Abstract

Purpose: To find out how spatial vision in mice is affected by wearing of spectacle lenses or diffusers, and by atropine eye drops. This information is necessary to determine which treatments could effectively induce refractive errors in young mice.

Methods: Whole-body optomotor responses were recorded by automated video analysis in freely ranging mice in a large rotating drum that was covered inside with vertical square-wave gratings with spatial frequencies of 0.03, 0.10 and 0.30 cyc/deg, both at "dim light" (0.10 cd/m^2), and under photopic conditions (30 cd/m^2). Contrast thresholds were determined by varying the contrasts of the gratings. Mice wore either no lenses, or binocular plano lenses, or lenses with powers ranging from +25 D to −25 D, or diffusers. In another experiment, contrast thresholds were determined 30 min after binocular installation of one drop of 1% atropine solution which is known to suppress myopia development in other animal models.

Results: The range of spatial frequencies, at which the mice still responded to stripes with less than the maximal grating contrast, was rather small. At 0.03 cyc/deg, the mice responded to stripes with low contrast down to 24%. At 0.10 cyc/deg, the minimal contrast was 45%, but at 0.30 cyc/deg, only the maximum contrast elicited a significant response. In dim light, spatial vision was severely impaired and only the lowest spatial frequencies, presented at the highest contrast (91%), were detected. The whole-body optomotor response was largest with spectacle lens powers of plano diopters and +7 D lenses. The magnitude of the response decreased symmetrically with increasing lens powers for both signs, providing information on the behavioral depth of field (a second-order fit through the data placed the extreme limits of a response at around +25 D and −25 D lens powers). Finally, atropine improved contrast sensitivity, at least at the lowest spatial frequency tested, a result that was previously obtained also in the chicken and could help to explain the inhibitory effect of atropine on myopia.

Conclusions: The study shows that mice have sufficient spatial vision to respond to treatment with powerful spectacle lenses or diffusers. Accordingly, these devices should be effective in inducing refractive errors in this animal model, although primarily under photopic conditions.

Keywords: Mouse; Myopia; Whole-body optomotor response; Contrast threshold; Atropine

1. Introduction

Refractive errors can be induced in animal models by covering their eyes with diffusers or spectacle lenses (review: Wallman & Winawer, 2004). However, a basic requirement is that the retinal image is sufficiently blurred to trigger the release of growth signals from the retina. In animals with high acuity and good optics, it is clear that even a low power lens produces detectable blur. This is not so clear in an eye with low optical quality and low visual acuity, like the mouse eye. The mouse was recently proposed as a new model for myopia studies (Fernandes et al., 2004; Schaeffel, Burkhardt,

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doi:10.1016/j.visres.2005.04.015

Howland, & Williams, 2004; Schmucker & Schaeffel, 2004; Tejedor & de la Villa, 2003), but it has to be demonstrated that diffusers or spectacle lenses produce significant changes in the contrast sensitivity of these animals. In the mouse, deprivation myopia develops only slowly (Fernandes et al., 2004; Schaeffel et al., 2004; Schmucker & Schaeffel, 2004; Tejedor & de la Villa, 2003) and a possible explanation is that the natural optical quality of the eye is so poor that diffusers degrade the retinal image only little further. Also, if the depth of focus would be as large as proposed by Remtulla and Hallett (1985) (±14 D), spectacle lenses with low power would scarcely affect spatial vision.

In a second set of experiments, we studied the effects of topical atropine on spatial vision in the mouse. The non-selective muscarinic antagonist atropine is currently the most potent drug against myopia development in both humans (Bedrossian, 1979; Chua, Balakrishnan, Tan, Chan, & ATOM study group, 2003; Gimbel, 1973) and animal models (chicks: Diether et al., 2004; McBrien, Moghaddam, & Reeder, 1993; monkeys: Raviola & Wiesel, 1985; Tigges et al., 1999). However, the mechanism by which myopia is suppressed is still unknown. A possible explanation is that atropine increases the contrast sensitivity of the retina, simulating a better image and reducing the error signal that would normally make the eye grow faster. In fact, it has been shown before that contrast sensitivity in chickens is increased after they had received an intravitreal injection of atropine (Diether & Schaeffel, 1999).

Recent studies have shown that visual acuity, contrast sensitivity and color vision can be measured in mice using behavioral paradigms (optomotor response: Abdeljalil et al., 2005; Prusky, Alam, Beekman, & Douglas, 2004; Schmucker, Seeliger, Humphries, Biel, & Schaeffel, 2005; Sinex, Burdette, & Pearlman, 1979; forced-choice procedures: Gianfranceschi, Fiorentini, & Maffei, 1999; Jacobs, Williams, & Fenwick, 2004; Prusky, West, & Douglas, 2000; Prusky, Reidel, & Douglas, 2000; Prusky & Douglas, 2003, 2004). These studies have shown, for instance, that grating acuity in the mouse is largely determined by the rod system (Schmucker et al., 2005), that it increases with illuminance (Abdeljalil et al., 2005; Schmucker et al., 2005) and that mice can make dichromatic color discriminations (Jacobs et al., 2004). The studies have also clarified how spatial acuity and contrast sensitivity develops with age (Prusky et al., 2004) and how this development is affected by ablation of the striate cortex (V1) (Prusky & Douglas, 2004). Also the effects of environmental enrichment (Prusky, Reidel, et al., 2000) and visual deprivation (Prusky & Douglas, 2003) on visual acuity were studied.

To determine how diffusers, spectacle lenses and atropine affect spatial vision in the mouse, we determined the contrast thresholds at different spatial frequencies with lenses or diffusers, or after topical application of eye drops with atropine. Measurements were performed under both photopic conditions and in dim light, using an optomotor paradigm that was recently developed (Schmucker et al., 2005).

2. Material and methods

2.1. Animals

All experiments were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The mouse experiments were approved by the University commission for animal welfare (reference AK3/03). The behavioral study included 31 black juvenile C57BL/6 wildtype mice. Ages ranged between 35 and 45 days. Black C57BL/6 wildtype mice were obtained from Charles River GmbH, Sulzfeld, Germany, and bred in the animal facilities of the Institute. The strains were completely inbred and, with the exception of sex chromosome differences and rare spontaneous mutations, all individuals were isogenic.

Animals were housed with their mothers until weaning at around day P21, and then in groups of three to four in standard mouse cages under a 12 h light/dark cycle. Ambient illuminance was provided by incandescent lights and was about 500 lux on the cage floor (measured with a calibrated photo cell in photometric mode). All experimental procedures were conducted under the light phase (between 10 a.m. and 4 p.m.) of the daily cycle.

2.2. Optomotor experiment

Contrast thresholds were evaluated in a whole-body optomotor experiment as previously described (Schmucker et al., 2005). In brief, mice were individually placed in a clear transparent acrylic glass cylinder (diameter: 15 cm; height: 18 cm) that was placed in the middle of a large rotating optomotor drum (diameter: 63 cm; height: 35 cm). The rotating drum was covered inside with vertical square-wave patterns with adjustable spatial frequencies. Angular speed of the stripe pattern was 50 deg/s. To keep the average brightness of the stripe patterns independent of their contrast (Michelson contrast), the gray levels of the darker stripes were lightened by a similar amount as the intermittent light stripes were darkened. The stripe patterns were printed on clear plastic foil using a 1200 dpi black and white laser printer. These foils were attached to the inner wall of the drum which was white painted. Stripe contrast was determined by direct measurements of stripe luminance by a luminance meter (LS-100 LS-110; Minolta, Osaka, Japan). It was focused either on the dark or the bright stripes and contrast was calculated by

$$C = (L_{max} - L_{min})/(L_{max} + L_{min})$$

with C the contrast, L the luminance of the stripes. Contrast is presented below in percent ($C * 100$).

The spatial frequencies of the stripe patterns assume that the fundamental was the limiting Fourier component. This can be assumed because the second harmonic for a square-wave grating is three times the fundamental frequency. This (1.50 cyc/deg) would be beyond what mice have ever have been proven to see (maximally 0.50–0.60 cyc/deg, e.g., Prusky, West, et al., 2000). Therefore, higher-order harmonics can be ignored.

Since the mice were not restrained and could freely move in the acrylic glass container, the angular subtense of the stripes changed according to their changing viewing distances, and accordingly the spatial frequency decreased when they approached the stripe pattern. The range of variability of the spatial frequencies tested was between 0.02 and 0.04 cyc/deg at 0.03 cyc/deg (the lowest frequency tested), between 0.08 and 0.12 cyc/deg at 0.10 cyc/deg (the spatial frequency at which the mice displayed the best responses in a previous study, Schmucker et al., 2005) and between 0.23 and 0.36 cyc/deg at 0.30 cyc/deg (the highest spatial frequency at which the mice showed significant responses, Schmucker et al., 2005).

Since it is not possible to judge reliably by eye whether the mouse followed a stripe pattern or not (Schmucker et al., 2005), the mice were imaged by a monochrome miniature surveillance video camera (PAL format, 752×536 Pixels, Conrad Electronics, Hirschau, Germany) that was centered in the top of the cylinder. Mice were tracked by a self-written video image processing program at 25 Hz frame rate. The program followed the movement of the mouse by determining the angular speed of the center of mass of the mouse body with respect to the center of the drum (“running speed”). Because the mouse also turned its snout-tail axis in response to the drifting stripes, its angular orientation speed was also evaluated as a second parameter (“orientation speed”). The “locomotor activity” was also recorded as the average absolute angular speed of the mouse. The baseline noise in the measured parameters was assessed (i.e., the response of the animals when no visual stimulus was present) in a previous study and was on average 0.0068 ± 0.0551 deg/frame for angular running speed and 0.0004 ± 0.0539 deg/frame for angular orientation speed (refers to both the response to a stationary drum and to a rotating drum; Schmucker et al., 2005).

During testing, the different spatial frequency gratings and contrasts were exchanged in a random order. Furthermore, the direction of ration of the drum was reversed approximately every 20 s and the reversion was repeated five times in each direction. The initial direction of rotation was randomly chosen. Angular running speed, angular orientation speed and locomotor activity were recorded for each direction of rotation of the drum. The unit for the measurements of the angular body movements was “degrees per frame”, with one frame lasting 40 ms (sampling frequency 25 Hz, European video format PAL).

2.3. Measurements under photopic conditions

Optomotor experiments were performed at an average luminance of the stripe patterns of 30 cd/m^2, as measured from the center of the acrylic glass cylinder at the level of the mouse eyes. A standard 60 W light bulb (Philips, Eindhoven, The Netherlands) served as light source. It was placed above the cylinder at a distance of 48 cm from the mouse.

Contrast thresholds were evaluated in 12 mice at the spatial frequencies described above (0.03, 0.10 and 0.30 cyc/deg). At spatial frequencies of 0.03 and 0.10 cyc/deg, the stripe patterns were presented with grating contrasts of 91%, 67%, 45%, 24% or 16%. At a spatial frequency of 0.30 cyc/deg, grating contrasts were 91%, 67%, 45% or 24%.

2.4. Measurements at dim light

An average luminance of the stripe pattern of 0.10 cd/m^2 was generated by a white LED (diameter 10 mm, mcd typ 1200; Conrad Electronics), that was also placed above the cylinder at a distance of 48 cm from the mouse. A frosted plastic diffuser was placed below the LED to provide a largely homogenous illumination. Spatial frequencies of 0.03 and 0.10 cyc/deg were tested at 91%, 67%, 45% or 24% grating contrast in seven mice. Mice were dark adapted for at least 60 min before the measurements were performed.

2.5. Measurements with spectacle lenses

To evaluate the effects of defocus on contrast sensitivity, 10 mice were tested under photopic conditions at a spatial frequency of 0.03 cyc/deg and maximum contrast (91%). During the optomotor experiment, spectacle lenses were attached to the eyes. Spherical PMMA lenses (obtained from HECHT Contactlinsen, Freiburg, Germany), with a diameter between 10.0 and 12.2 mm and a radius between 7.8 and 8.4 mm, were used. The rims of the lens, about 1 mm wide, were attached to the fur around the eyes with ring-shaped double-sided tape (one side: adhesive tape; the other side: Velcro®) with an inner diameter of about 9 mm and an outer diameter of about 13 mm (obtained from Schell Naehzubehoer, Aachen, Germany). The lenses did not interfere with the normal functions of the eyelids. To prevent that the mice could remove the lenses during their cleaning behavior, plastic collars were fitted

around their necks as previously described (Schaeffel et al., 2004). Before the measurements, the mice were adapted to the collars for at least 24 h. Lenses were attached 20–30 min before the optomotor experiment started under light ether anesthesia. The same lens powers were used in both eyes. The tested lens powers were +7 D, +25 D, −8 D, −15 D and −25 D. As controls, plano lenses were also tested.

2.6. Measurements with diffusers

Four mice were tested while their vision was blurred with hand-made frosted hemispherical thin plastic shells that were previously used to induce deprivation myopia in mice (Schaeffel et al., 2004; Schmucker & Schaeffel, 2004). The contrast modulation transfer of the diffusers was measured by imaging a stripe pattern with a video camera with and without diffuser foil in front of the camera lens. It turned out that the contrast modulation transfer was close to zero at the tested spatial frequencies of 0.03, 0.10 or 0.30 cyc/deg, indicating that no spatial vision was possible. Accordingly, no behavioral responses were expected in the drum experiment. Diffusers were attached around the mouse eye in the same way as the spectacle lenses, and plastic collars were applied as described above. Diffusers were tested under photopic conditions.

2.7. Measurements with topical atropine

One drop of atropine (1% solution) was instilled in both eyes of five mice. A drop had a measured volume of 33 μl, and contained 330 μg atropine sulfate. In mice, like in humans, atropine causes a wide dilation of the pupil. Therefore, pupil size which also effects retinal image brightness was studied by video pupillography (Schaeffel & Burkhardt, 2005), before the optomotor experiments, about 20 min after atropine instillation. The change in retinal brightness was calculated with the formula: (pupil diameter without atropine)^2/(pupil diameter after topical atropine)^2. Contrast thresholds were measured under photopic conditions, at spatial frequencies of 0.03, 0.10 or 0.30 cyc/deg, and using the same contrasts as described above.

2.8. Statistics

The *response* (whole-body optomotor response) of the mouse was defined as the difference of its average angular movement speed (considering the algebraic sign of the direction of the movement) when the drum was rotating clockwise versus counter clockwise. These differences were analyzed both for the angular running speed and angular body orientation speed. The more different this value was from zero or the more it differed from the response when no visual stimulation occurred (evaluated in a previous study, Schmucker et al., 2005 and also see above) the more important the visual input was to the mouse behavior.

Mean responses and standard deviations were plotted against grating contrast or, in case of the lens experiment, against lens power. To estimate the contrast threshold (the lowest contrast that elicited a significant response), the responses were tested against zero, using an unpaired one-sample t test. Additionally, responses at different grating contrasts and responses with lenses and diffusers were tested against the response when no visual stimulation occurred, using an analysis of variance (one-way ANOVA). Post hoc analysis (the Dunnett test) was performed on factors that were found to be significant in the ANOVA. The significance level was set at 5%.

To test whether the treatments interfered with mouse activity, variance ratio tests were used to compare the locomotor activity of diffuser, lens or atropine-treated mice to that of untreated mice. Statistical tests were performed on computer (JMP, version 4 software; SAS Institute, Cary, NC).

3. Results

3.1. Contrast thresholds under photopic conditions

Average whole-body optomotor responses for three spatial frequencies and their standard deviations at different grating contrasts are shown for both angular running speed and orientation speed in Fig. 1. There was no significant difference between angular running speed and angular orientation speed (difference: 0.00 ± 0.04 deg/frame, $P = 0.10$, unpaired one-sample t test).

The largest responses were obtained at the lowest spatial frequency tested (0.03 cyc/deg). At this spatial frequency, mice displayed a significant response at 91%, 67%, 45%, and 24% contrast. Below 24% contrast, more animals began to move randomly ($P > 0.05$, unpaired one-sample t test). At a spatial frequency of 0.10 cyc/deg, the threshold was already reached at 45% contrast. At this spatial frequency, no significant response could be elicited at lower contrasts ($P > 0.20$, unpaired one-sample t test). At the highest spatial frequency tested (0.30 cyc/deg), significant responses were measured only at the maximum possible contrast ($P = 0.03$, unpaired one-sample t test). In addition, comparing the responses to the condition when no visual stimulation occurred (evaluated in a previous study, on average 0.01 ± 0.06 deg/frame for angular running speed and 0.00 ± 0.05 deg/frame for angular orientation speed; Schaeffel & Burkhardt, 2005), significance difference was revealed ($P < 0.01$, one-way ANOVA). The contrast thresholds found by one-sample t tests were confirmed by a post hoc analysis ($P < 0.05$, Dunnett test).

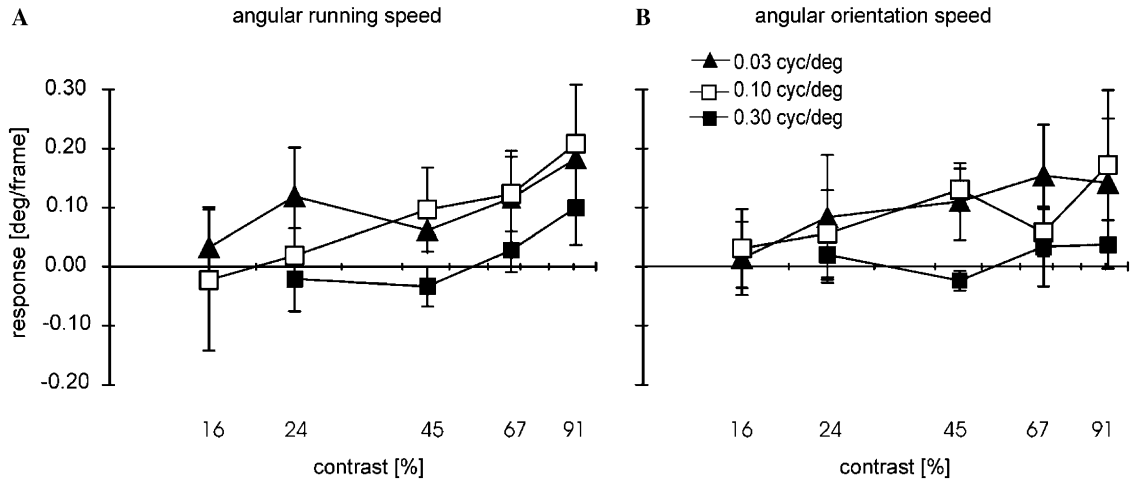

Fig. 1. Whole-body optomotor responses and their standard deviations of C57BL/6 mice at three spatial frequencies. Responses are plotted against grating contrast, for both angular running speed (A) and angular orientation speed (B). Average luminance of the stripes was 30 cd/m^2. Data on responses at maximum contrast (91%) originate from Schmucker et al. (2005). Averages from 12 animals are shown, with four or more animals tested at each data point. Responses were significantly different from zero ($P < 0.05$, unpaired one-sample t test) and from the response when no visual stimuli was present (evaluated in a previous study by Schmucker et al., 2005; $P < 0.05$, Dunnett test) at 24% contrast or higher at a spatial frequency of 0.03 cyc/deg, at 45% or higher at 0.10 cyc/deg and only at 91% at 0.30 cyc/deg.

3.2. Contrast thresholds in dim light

Fig. 2 shows the whole-body optomotor responses of the mice at a luminance of 0.10 cd/m^2, for spatial frequencies of 0.03 and 0.10 cyc/deg. In these experiments, angular running speed was slightly higher than angular orientation speed (difference: 0.03 ± 0.03 deg/frame, $P = 0.05$, unpaired one-sample t test). In dim light, significant responses were only obtained at the maximum stripe contrast, both at 0.03 and 0.10 cyc/deg ($P < 0.02$, unpaired one-sample t test). At lower contrasts, the movements of the mice in the drum were random ($P > 0.20$, unpaired one-sample t test). Comparing the responses at 0.10 cd/m^2 with the response when no visual stimuli were present, a one-way ANOVA showed significant differences ($P = 0.001$). The Dunnett test showed that only the whole-body response at 0.10 cyc/deg with maximum contrast reached significance ($P < 0.05$).

3.3. Contrast thresholds with spectacle lenses

Fig. 3 shows whole-body optomotor responses of mice to a grating with 0.03 cyc/deg and with 91%

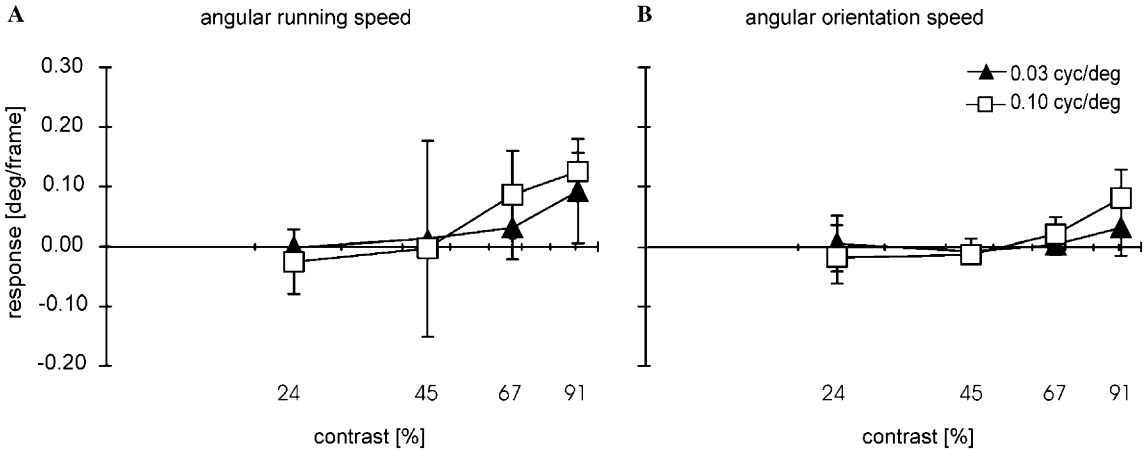

Fig. 2. Whole-body optomotor responses in dim light (0.10 cd/m^2). Angular running speed (A) and angular orientation speed (B) are shown at two spatial frequencies. Data on the responses at maximum contrast (91%) originate from Schmucker et al. (2005). Data from seven animals are shown, with three or more animals tested at each data point. Comparing the responses in dim light against the null hypothesis, significance was reached only at the highest contrast at both spatial frequency ($P < 0.05$, unpaired one-sample t test). If compared with the response when no visual stimulation was present (evaluated in a previous study by Schmucker et al., 2005), only the response at 0.10 cyc/deg with maximum contrast was significant ($P < 0.05$, Dunnett test).

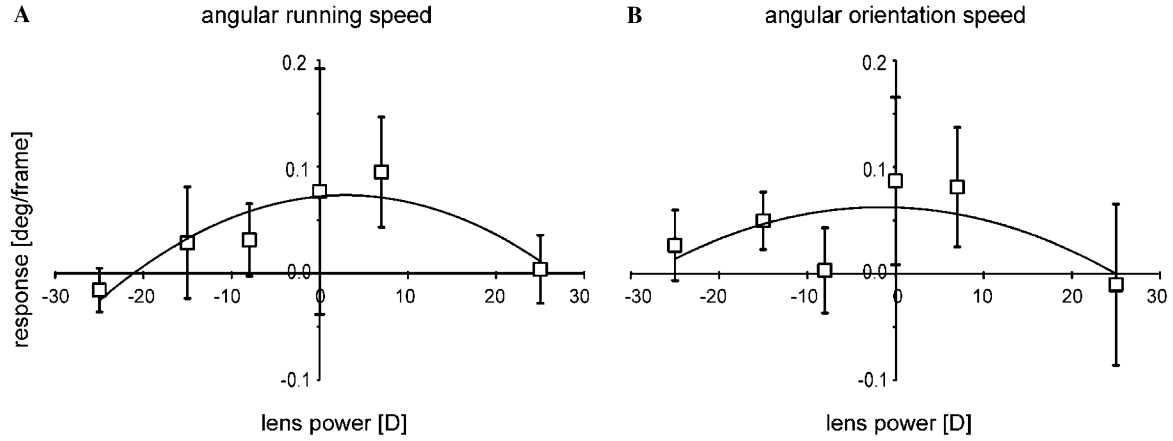

Fig. 3. Whole-body optomotor responses with equal power spectacle lenses in front of both eyes, for angular running speed (A) and angular orientation speed (B). The data points of the plano lenses, +7 D and −8 D lenses show the mean responses and standard deviations from four animals. The data points of the −15 D, −25 D and +25 D lenses show the mean responses and standard deviations from five animals. Measurements were performed at a spatial frequency of 0.03 cyc/deg with a contrast of 91%, under photopic conditions. The whole-body optomotor responses decreased symmetrically in both directions with increasing defocus and were described by an inverted parabola (second-order polynomial fit). The equations were $y = -0.0001x^2 + 0.0007x + 0.0722$, $R^2 = 0.8348$ in the case of angular running speed and $y = -9E{-}0.5x^2 + 0.0003x + 0.0621$, $R^2 = 0.4249$ in the case of angular orientation speed.

contrast, when they were wearing spectacle lenses of various powers. In these experiments, no significant difference was reached between angular running speed and angular orientation speed (difference: 0.00 ± 0.02 deg/frame, $P = 0.80$, unpaired one-sample t test).

The largest responses were found when the mice had either plano and +7 D lenses (Fig. 3; mean angular running speed: 0.08 ± 0.12 deg/frame and 0.09 ± 0.05 deg/frame, respectively; mean angular orientation speed: 0.09 ± 0.08 deg/frame and 0.08 ± 0.06 deg/frame, respectively). However, only the responses with the +7 D lenses achieved significance ($P < 0.05$, unpaired one-sample t test). With −15 D lenses, the mice were still able to resolve the grating (angular orientation speed: 0.05 ± 0.03 deg/frame; $P = 0.01$, unpaired one-sample t test). With +25 D or −25 D lenses, the whole-body optomotor responses were not different from zero ($P > 0.16$, unpaired one-sample t test).

Additionally, a one-way ANOVA was performed to identify differences between the responses with lenses and the response without visual stimuli ($P = 0.03$). The post hoc analysis showed that there was a difference between the response with plano lenses and the response when no visual stimulation occurred in the case of angular orientation speed ($P < 0.05$, Dunnett test).

To obtain "pooled information" about the behavioral depth of field, the data on the whole-body optomotor responses with different lens powers were fit with an inverted parabola (second-order polynomial fit), providing the following equations: for angular running speed: $y = -0.0001x^2 + 0.0007x + 0.0722$, $R^2 = 0.8348$; for angular orientation speed: $y = -9E{-}0.5x^2 + 0.0003x + 0.0621$, $R^2 = 0.4249$. The parabolas show that the response was maximal at about zero diopter lens power, and declined symmetrically in both direction with increasing defocus. The fits intersect with the abscissa at about +25 D and −25 D, respectively, indicating that definitely no response was left at such high amounts of defocus.

Locomotor activity was significantly increased in mice wearing spectacle lenses (0.29 ± 0.03 deg/frame vs. 0.20 ± 0.02 deg/frame; $P = 0.001$, variance ratio test). The increase in activity could either be due to the attempts of the mice to remove the lenses, or due to light ether anesthesia which was necessary to attach the lenses about 30 min before the measurements started.

3.4. Contrast thresholds with diffusers

Fig. 4 shows the whole-body optomotor responses of mice wearing translucent diffusers over both eyes. There was no significant difference between angular running speed and orientation speed (difference: 0.00 ± 0.05 deg/frame, $P = 0.82$; unpaired one-sample t test). The optomotor experiment confirmed our expectations and showed that spatial vision was largely abolished since the whole-body optomotor responses became random ($P > 0.05$, unpaired one-sample t test). Comparing the responses of mice wearing diffusers with the response without visual stimulation, no significant difference was revealed ($P > 0.05$, one-way ANOVA).

As with the spectacle lenses, the locomotor activity increased at 0.03 cyc/deg (0.36 ± 0.04 deg/frames vs. 0.20 ± 0.02 deg/frames, $P = 0.001$, variance ratio test). However, there was no significant increase at 0.10 and 0.30 cyc/deg ($P = 0.10$, variance ratio test). Since significant whole-body optomotor responses were observed in the case of the lenses (Fig. 3), the lack of responses

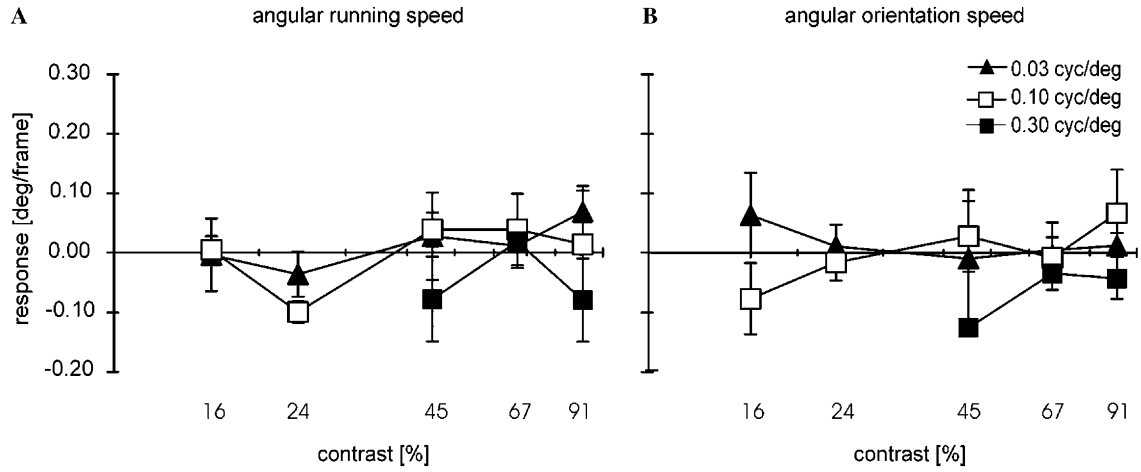

Fig. 4. Whole-body optomotor responses with the heavily frosted diffusers in front of both eyes, measured at different grating contrasts. Angular running speed (A) and angular orientation speed (B) were measured at a luminance of 30 cd/m^2. Data from four animals are shown, at least three mice were tested at each data point. Responses were not significantly different from zero ($P > 0.05$, unpaired one-sample t test) and not different from the response when no visual stimulation occurred (evaluated in a previous study by Schmucker et al., 2005; $P > 0.05$, one-way ANOVA), indicating that spatial vision was largely abolished.

cannot be attributed to extensive cleaning behavior and neglect of the presented stripe patterns.

3.5. Contrast thresholds after atropine eye drops

Pupil diameters after atropine application were 2.02 ± 0.07 mm (approximately double of the pupil diameter at a luminance of 30 cd/m^2 which is about 1 mm, Garcia de la Cera et al., 2005). The increase in pupil size increased the retinal image brightness by a factor of 4, equivalent to 0.3 log units.

Whole-body optomotor responses for three spatial frequencies and different grating contrasts are shown in Fig. 5. There was no significant difference between angular running speed and angular orientation speed (difference: 0.01 ± 0.04 deg/frame, $P = 0.41$, unpaired one-sample t test).

Surprisingly, but in line with previous findings in chickens (Diether & Schaeffel, 1999), atropine increased the contrast sensitivity, at least, at the lowest spatial frequency that was tested (0.03 cyc/deg). Here, the mice displayed significant whole-body responses down to contrasts of 16% ($P = 0.02$, unpaired one-sample t test). At higher spatial frequencies, the contrast thresholds were similar to the thresholds in untreated mice, measured under photopic conditions (at 0.10 cyc/deg the

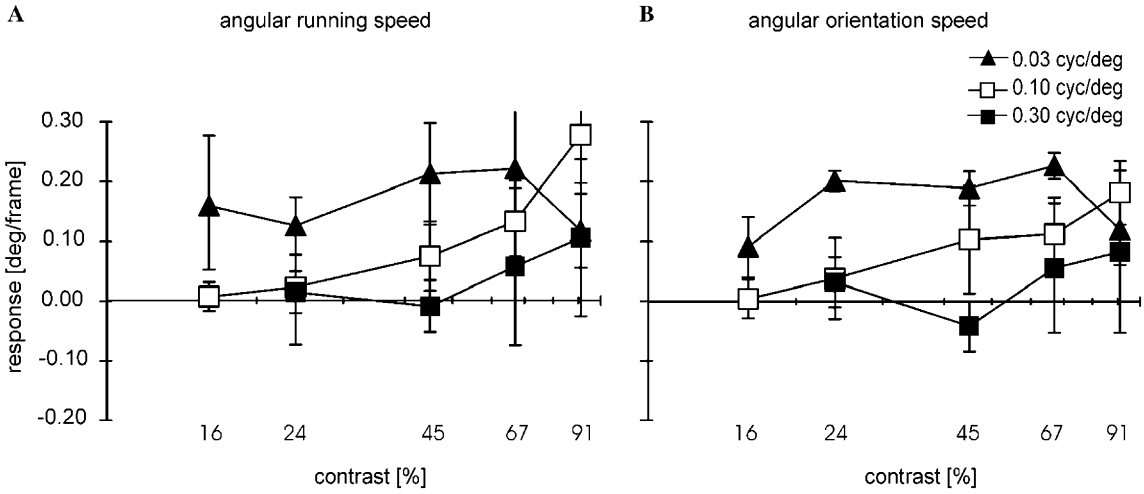

Fig. 5. Whole-body optomotor responses after application of atropine. Angular running speed (A) and angular orientation speed (B), measured at a luminance of 30 cd/m^2, are plotted against grating contrast. Data from five animals are shown, with three or more animals tested at each data point. Significant whole-body optomotor responses against the null hypothesis ($P < 0.05$, unpaired one-sample t test) and against the response when no visual stimuli was present (evaluated in a previous study by Schmucker et al., 2005; $P < 0.05$, Dunnett test) were elicited down to grating contrasts of 16%, at a spatial frequency of 0.03 cyc/deg, of 45% at 0.10 cyc/deg, but only of 91% at 0.30 cyc/deg.

threshold was 45% and at 0.30 cyc/deg the threshold was 91%, $P < 0.03$, unpaired one-sample t test). In addition, a one-way ANOVA was performed and revealed that the responses were different from the response without visual stimuli ($P < 0.03$). The conclusions drawn by the unpaired one-sample t test were confirmed by a post hoc analysis ($P < 0.05$, Dunnett test).

It was excluded that the increase in contrast sensitivity was due to increased locomotor activity. At the lowest spatial frequency tested, locomotor activity was only slightly increased, compared to untreated mice but this difference did not achieve significance (0.24 ± 0.03 deg/frame vs. 0.20 ± 0.02 deg/frame, $P = 0.07$, variance ratio test). At the other spatial frequencies tested, there was not even a trend to an increase of the locomotor activity ($P > 0.91$, variance ratio test).

4. Discussion

The study shows that the mice had sufficient spatial vision to respond to treatment with spectacle lenses or diffusers. Accordingly, these treatments should be effective in inducing refractive errors in this animal model since they degrade the retinal image quality by a detectable amount. The study also shows that the range of spatial frequencies at which the mouse responded to changes in contrast was rather small. Only at 0.03 cyc/deg, the mice responded to stripes with low contrast down to 24%. At 0.30 cyc/deg, only the maximum contrast (91%) elicited a significant whole-body optomotor response. These results suggest that the contrast sensitivity function, as measured in a whole-body optomotor paradigm, should not extend far beyond 0.30 cyc/deg. In dim light, spatial vision was severely impaired and only the lowest spatial frequencies, presented at the highest contrast, were detected. Finally, atropine appeared to improve contrast sensitivity, a result that was previously obtained also in chickens (Diether & Schaeffel, 1999).

4.1. Comparisons to contrast thresholds measured in previous studies

In the present study, the measured contrast thresholds in mice with normal vision were generally higher than in other studies (e.g., visual water task: Prusky & Douglas, 2004; optomotor response: Prusky et al., 2004; electrophysiological measurements: Porciatti, Pizzorusso, & Maffei, 1999). In our study, the threshold contrast was about 24% at the lowest spatial frequency tested (0.03 cyc/deg), and increased to 45% at 0.10 cyc/deg and to 91% at 0.30 cyc/deg. In a recent study by Prusky et al. (2004), the peak contrast sensitivity was found at a spatial frequency of 0.064 cyc/deg in mice at the age of 30 days (maximum sensitivity: 24.5 or contrast threshold: 4%), using a virtual optomotor drum. In this study, reflexive head movements were tracked in unrestrained mice while they were facing a rotating three-dimensional vertical sine-wave grating presented on four computer monitors that formed a square. Furthermore, in this study, the contrast sensitivity decreased with increasing spatial frequencies as in our study, but less so (at 0.10 cyc/deg, the contrast threshold was at 16% contrast; at 0.272 cyc/deg, it was at 25%). Using the visual water task (Prusky & Douglas, 2004), the contrast sensitivity curve peaked at 0.208 cyc/deg with a contrast threshold of 15.7%. As in the present study, contrast thresholds increased with increasing spatial frequencies (at 0.50 cyc/deg, the contrast threshold was at 50%). Porciatti et al. (1999) used visually evoked potentials to evaluate contrast sensitivity in mice. They found a contrast threshold of 5% for coarse gratings (0.06 cyc/deg) and of 17% for fine gratings (0.20 cyc/deg).

Why the contrast thresholds were higher in our whole-body optomotor experiments, compared to other studies, could be explained either by differences in the visual system of the different laboratory mouse strains (which appears less likely), or by differences in the behavioral testing paradigms. Measurements of the "whole-body optomotor response" generate probably more variability than measurements of optokinetic eye movements or optomotor responses of the head, in particular in animals with small eyes and large heads like in mice. Therefore, the noise in the behavioral data presented here is probably larger than in previous studies that analyzed the movements of the eyes or the head since spontaneous activity is superimposed in all the tracking records. On the other hand, a clear advantage of our technique is that the animals are restrained only by the walls of the container, and that the analysis is automated and not affected by observer bias.

A further limitation might be that acuity and depth of focus of the optokinetic/optomotor system are determined by the population of directionally sensitive retinal ganglion cells that project to the nucleus of the optic tract in the pretectum and thus would potentially not be the same as that of the emmetropization system.

That the experimental procedures introduce different levels of noise was already stated by Prusky et al. (2004), who recognized that the spatial frequency threshold was lower in their virtual optomotor experiment than in other psychophysical experiments.

4.2. Contrast thresholds in dim light

To our knowledge, contrast thresholds in dim light have not been studied before. Our study shows that, at 0.10 cd/m^2 (where only rod vision is possible; Schmucker et al., 2005), gratings were detected only at maximum contrast (91%). No spatial vision was detected at a contrast of 67%, or below. To find out whether differences

in the spectral composition of the light source could account for some of the behavioral differences, the spectra of the white LED and standard 60 W light bulb were analyzed with a hand-held spectroscope. Both light sources had continuous spectra with only a minor difference in the bandwidth (white LED: 425–620 nm, maximum at 450 nm; 60 W bulb: 400–650 nm, maximum at 560 nm). Therefore, it is obvious that these small differences cannot account for the observed difference in whole-body optomotor responses in dim and bright light.

The results presented here suggest that experiments with diffusers to study deprivation myopia in mice should not be successful in dim light. The underlying assumption is that induction of deprivation myopia requires an alteration of spatial vision. It has been shown in other animal models that deprivation of spatial frequencies and contrast, but not of light, is the most effective way to induce deprivation myopia (Feldkaemper, Diether, Kleine, & Schaeffel, 1999). Given that mice show little spatial vision at low light, diffusers cannot induce any further changes in spatial vision. Accordingly, the conclusion is deprivation myopia can probably not be induced with diffusers at low light levels.

4.3. Refractive state inferred from optomotor experiments with lenses

The data on the whole-body optomotor responses with different lenses provide – for the first time – some information on the behavioral depth of field in mice.

The decline in response was about symmetrical with respect to refraction zero (Fig. 3; the decrease in response with increasing lens power could be described by an inverted parabola), suggesting that the average refractive state was close to zero diopters. The fits intersect with the abscissa at about −25 D and +25 D. These are probably the upper and lower limits of any responses and denote the extreme limits of the behavioral depth of field. The large standard deviations obtained in this behavioral experiment can be explained either by the fact that these animals were wearing lenses in the optomotor drum and were, therefore, not very cooperative because they tried to remove their lenses or by the restricted field of view due to the lenses. The literature provides slightly lower values for the depth of field: in the pattern electroretinogram (Porciatti, Pizzorusso, Cenni, & Maffei, 1996) and in visual evoked potentials (Porciatti et al., 1999), trial lenses in front of the eyes of ±10 D in power did not alter the response amplitudes.

The fact that the stripe patterns were not presented at infinity but rather at about 3 D (drum radius: 31.5 cm) is of minor importance. A difference of 3 D in refraction cannot be resolved in Fig. 3. The true subjective refractive state of the mouse was evaluated in a previous paper (Schmucker et al., 2005). In this study, spatial acuity was tested in two drums with different size (a large drum with a diameter of 63 cm (equivalent to about 3 D) and a small drum with a diameter of 22 cm (equivalent to about 10 D)). There was a slight improvement in spatial acuity when the smaller drum was used (large drum: 0.30 cyc/deg, small drum: 0.50 cyc/deg). This observation could suggest that the mice were slightly myopic or that the mice approached the stripe patterns, increasing the viewing angle and reducing spatial frequencies. The latter was more likely, because the introduced variability of the spatial frequencies in the small drum was between 40% and 65%. Moreover, since small eyes with high refractive power have a large dioptric depth of focus (Green, Powers, & Banks, 1980) the gratings were probably in best focus in both drums, and the potential myopia was not limiting.

4.4. Contrast thresholds after atropine eye drops

Surprisingly, despite the larger pupil diameter which should result in a decline in optical quality of the eye, the contrast threshold was significantly lowered, at least, at 0.03 cyc/deg ($P < 0.05$, Dunnett test). It could be possible that contrast sensitivity was increased only because the retinal image was brighter due to the dilatation of the pupil with atropine and this optical effect is not related to functional changes in the retina. Pupil sizes without atropine at the brightest luminance condition (about 30 cd/m^2) were about 1.0 mm and, under atropine treatment, they were about 2.0 mm. Doubling pupil diameter increased the retinal image brightness by a factor of 4, or 0.3 log units. In a previous experiment (Schaeffel & Burkhardt, 2005) it was found that a reduction of the luminance of the stripe pattern by 1.3 log units (from its initial value of 30 cd/m^2) reduced the behavioral response only by 24%. Therefore, the lower contrast threshold with atropine can probably not be explained only by the optical effects of the pupil. Furthermore, it is even likely that the mices' retinas were "overexposed" under cycloplegia and this should have caused saturation with reduced contrast sensitivity, rather than an improvement. The mechanisms by which atropine can enhance contrast sensitivity are subject to speculation. It was previously shown that atropine boosts the release of dopamine from the retina (Schwahn, Kaymak, & Schaeffel, 2000) and it is known that dopamine reduces receptive field sizes and can increase contrast sensitivity at higher spatial frequencies (Bodis-Wollner & Tzelepi, 1998). The observed change in contrast sensitivity is in line with an idea by Diether and Schaeffel (1999), derived from experiments in the chicken, that the increase in contrast sensitivity of the retina with atropine may reduce the error signal that is generated with diffusers and that results in a stimulation of axial eye growth.

Acknowledgments

This work was supported by the Fortune Program of the Medical Faculty of the University of Tuebingen (# 1230-0-0) and the German Research Council (Scha 518/11-1). We thank Eva Burkhardt for excellent technical assistance.

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